HOST-PARASITE RELATIONSHIPS AND INTRASPECIFIC VARIATION IN *POSTHODIPLOSTOMUM MINIMUM* (TREMATODA: DIPLOSTOMATIDAE)

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ABSTRACT.— *Posthodiplostomum minimum* (MacCallum 1921), a strigeoid trematode normally found in the intestine of piscivorous birds, has been shown to be capable of developing in 17 orders of amphibian, reptilian, avian, and mammalian hosts. Both *Physa gyrina* and *Lymnaea reflexa* serve as the first intermediate host. Only sunfish from the lakes region were infected with metacercariae of *P. minimum*, indicating the presence of two physiologically distinct strains of *Posthodiplostomum*. Extensive feeding experiments involving all four vertebrate classes of hosts demonstrated the lack of host specificity in this genus.

Posthodiplostomum minimum (MacCallum 1921) is a strigeoid trematode of the family Diplostomatidae Poirier 1886. Adults of this species (Fig. 1) parasitize the intestine of piscivorous birds, and the metacercarial stage is found in various freshwater fishes.

Two subspecies of *P. minimum* have been reported, based upon the ability of cercariae to penetrate and develop either in centrarchid or cyprinid fish hosts (Hoffman 1958).

Since Stunkard's report on intraspecific variation in 1957, several more recent experimental studies have shown that size, shape, and position of various organs and structures in helminths may be considerably modified by the host. For many years, investigators such as Dubois (1944, 1955, 1968, 1970) have delineated species of strigeoids largely on the basis of host specificity. Recently, however, several investigators have shown that parasites can, indeed, develop within hosts that normally would be ecologically isolated from involvement in the normal life cycle of the parasite.

HISTORICAL REVIEW

Early literature concerned with the taxonomy and development of *P. minimum* is confusing, principally because various cercariae and metacercariae have been associated with the adult stage. Two distinct subspecies or physiological strains, namely *P. minimum minimum* and *P. minimum centrarchi*, are now recognized.

Throughout the literature, five cercarial types have been reported and described as belonging to *Posthodiplostomum minimum*: *Cercariae multicellulata*, H. Miller, 1923, 1925; *C. physaei*, Cort and Brooks, 1928; *C. louisiana*, C. Miller, 1954; *C. minimum*, J. Miller, 1954; and *C. paramulticellulata*, Goodman, 1951. Because of the synonomy and inadequate descriptions of these cercarial types and because of reported differences in size, number, and arrangement of their setae and spines, flame cell patterns, tail stem musculature, and the presence or absence of caudal bodies, these reports must be viewed critically. Bedinger and Meade (1967) reported a sixth cercarial type (from *Physa halei*) said to be distinct from those reported above. Their study indicated that several physiological strains or subspecies of *P. minimum* appear to exist, but no subspecies designation was proposed for their specimen.

The neascus-type metacercaria of *P. minimum* has been by far the most studied stage in its life cycle. It is this stage that is so often reported in fish-parasite surveys throughout the United States.

Leidy (1856) reported *Diplostomum cuticola*, the species presently known as *Posthodiplostomum minimum*, from the integument of freshwater fishes. Adult *Diplostomum minimum* (=*P. minimum*) was first reported by MacCallum (1921) from the intestine of a great blue heron found dead at the Zoological Park in New York. Because of previous inadequate descriptions of the metacercaria of *P. minimum*, Agersborg (1926) described metacercariae from the blunt-nosed minnow as *Diplostomum vancleavi*, but in his description he confused anterior and posterior ends. Hughes (1928) redescribed

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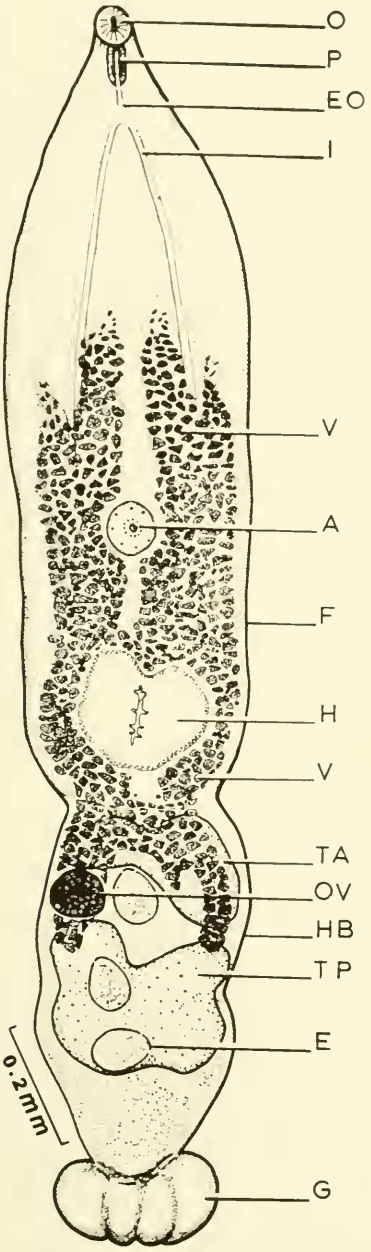

FIGURE 1. Diagram of adult *P. minimum* from the gull (*Larus argentatus*) depicting major organs undergoing morphological variation: A-acetabulum; E-egg; EO-esophagus; F-forebody; G-genital bursa; H-holdfast organ; HB-hindbody; I-intestine cecum; O-oral sucker; OV-ovary; P-pharynx; TA-anterior testis; TP-posterior testis; V-vitellaria.

this form as *Neascus vancleavi*. In 1936, based on studies of specimens from the intestine of a great blue heron, Noble renamed the adult *Neodiplostomum orchilongum*, which he considered as representing a new species. Dubois (1936), in a taxonomic study of the Strigeida, established the genus *Posthodiplostomum* and included in it members of the Diplostomatidae parasitic in birds of the family Ardeidae. He also reduced *D. minimum* to synonomy with *Posthodiplostomum minimum*. In 1937 Ferguson considered *N. orchilongum* as a synonym of *P. minimum*, an opinion confirmed by Dubois in 1938 in his monograph on the Strigeida.

The first report of possible host specificity of subspecies or of physiological strains of *P. minimum* was that of Klak (1940), who found metacercariae developing in two separate lines of fish, the Cyprinidae and Centrarchidae. Ferguson (1943) reconfirmed Klak's investigation and suggested that the taxonomic confusion surrounding *P. minimum* could be resolved only through experimental and morphological studies.

In summary, the synonyms reported for the adult fluke now considered to be *Posthodiplostomum minimum* are as follows:

Diplostomum minimum MacCallum, 1921
Neodiplostomum minimum (MaCallum, 1921) Dubois, 1935
Posthopilostomum minimum (MacCallum, 1921) Dubois, 1936
Neodiplostomum orchilongum Noble, 1936
Posthodiplostomum orchilongum (Noble, 1936) Dubois, 1937
Diplostomum vancleavi Agersborg, 1925
Diplostomum cuticola Leidy, 1856

Materials and Methods

The lakes region of northwest Iowa is an area rich in conditions requisite for the production of both snail and fish intermediate hosts of *Posthodiplostomum minimum*. It also serves as both a feeding and nesting area for piscivorous avian hosts needed in maintaining the life cycle of *P. minimum*. Fish, amphibian, reptilian, avian, and mammalian hosts used in experimental studies were taken from this area during 1971-1973. Additional hosts were acquired from local commercial hatcheries, from the Department of Genetics, and from the Department of Zo-

ology and Entomology at Iowa State University.

Intermediate hosts.— Laboratory-reared snails (*Physa gyrina* and *Lymnaea reflexa*) were maintained as described by Ulmer (1970). A diet of fresh lettuce, commerical fish food, and crushed oyster shells was provided.

Routine laboratory methods were utilized in isolation, examination, and identification of snails shedding larval stages of *P. minimum*. Dishes containing infected snails were examined twice daily for the presence of emerged *P. minimum* cercariae. Snails shedding such cercariae were isolated and transferred to one-gallon aquaria. Nonshedding snails were crushed and examined for developing sporocysts or placed in holding tanks for collection of egg masses to be used in the laboratory rearing of snails.

Sunfish (*Lepomis gibbosus* and *Lepomis macrochirus*) used for this study were collected with the aid of a hoop net or provided by the Iowa State Conservation Commission. All sunfish were transferred from a 20-gallon field container to large aquaria (50-100 gallons) within one hour after collection. Fish were maintained in doubly filtered lake water under constant aeration. A diet of commercially prepared fish food was fed to all fish each morning.

Fish (Table 1) used in surveying the extent of natural infection rate of *P. minimum* in West Lake Okoboji were examined within 36 hours.

Forty-one sunfish (*L. gibbosus* and *L. macrochirus*) seined from East Lake Okoboji (in a region known to be free from molluscan intermediate hosts) were used as a source of fish free from *P. minimum* infection. Careful examination of 19 of these sunfish indicated a complete absence of *P. minimum* metacercariae. The remaining 22 sunfish were maintained in a 100-gallon aquarium filled with flowing doubly filtered lake water under constant aeration. Water temperature was gradually lowered to 38 F, which aided in retarding fungal infections of the sunfish as well as in reducing the amount of commercial food needed to maintain these hosts.

Definitive hosts.— In preliminary controlled experiments hosts were collected from the wild and held in the laboratory for at least 15 days or more. Fecal smears were made of all hosts to determine if an infection of *P. minimum* existed.

Four classes of vertebrates (Amphibia, Reptilia, Aves, and Mammalia) were used as definitive hosts in this study. In preliminary investigations hosts were fed livers from sunfish containing naturally infected metacercariae of *P. minimum*. In additional experiments definitive hosts were fed portions of sunfish livers which had been experimentally infected with laboratory-developed *P. minimum* metacercariae. Once exposed, hosts were isolated in appropriate cages and given only water.

Amphibian hosts (Tables 2, 3) were force-fed infected sunfish livers containing over 100 naturally acquired *Posthodiplostomum minimum* metacercariae. Fecal material was examined for eggs to determine the presence of a previous infection of *P. minimum*. All hosts shown to be negative for trematode eggs were used for experimental feedings. All amphibians were maintained in a 20-gallon

Table 1. Fish examined for metacercariae (neascus) of *Posthodiplostomum minimum* in Lake West Okoboji.

Fish	Common name	Number collected	Number infected
Aplodinotus grunniens Rafinesque	Freshwater Drum	47	0
Cyprinus carpio Linnaeus	Carp	18	0
Esox lucius Linnaeus	Northern Pike	2	0
Ictalurus melas Rafinesque	Black Bullhead	26	0
Lepisosetus platostomus Rafinesque	Shortnose Gar	6	0
Lepomis gibbosus (Linnaeus)	Pumpkinseed	170	170
Lepomis macrochirus macrochirus Rafinesque	Bluegill	125	125
Perca flavescens (Mitchill)	Yellow Perch	25	0
Pomoxis nigromaculatus (Le Sueur)	Black Crappie	30	0
	Total	449	295

TABLE 2. Amphibian hosts fed sunfish livers naturally infected with metacercariae of *P. minimum*.

Hosts	Age of infection (hrs)	Host sex	Number of specimens recovered	State of sexual maturity
ORDER: URODELA				
Ambystoma tigrinum	72	♂	1-10	Mature
ORDER: ANURA				
Bufo americanus	96	♀	0	0
Rana Pipiens	84	♀	100+	Gravid
"	96	♀	1-10	Mature

TABLE 3. Amphibian hosts fed sunfish livers experimentally infected with metacercariae of *P. minimum*.

	Age of infection (hrs)	Host sex	Number of specimens recovered	State of sexual maturity	Laboratory maintenance of life cycle
ORDER: URODELA					
Eurycea bislineata	72	♂	1-10	Gravid	
"	72	♀	1-10	Mature	
"	72	♂	1-10	Mature	
"	72	♀	0	0	
"	72	♀	0	0	
Ambystoma tigrinum	72	♂	50-100	Mature	
"	72	♂	25-50	Gravid	
"	72	♀	50-100	Mature	
"	72	♂	1-10	Mature	
"	72	♂	1-10	Mature	
"	72	♀	0	0	
"	72	♀	0	0	
"	72		0	0	
ORDER: ANURA					
Bufo americanus	72	♂	25-50	Mature	
"	72	♂	1-10	Mature	
"	72	♀	0	0	
"	72	♀	0	0	
"	72	♀	0	0	
Rana pipiens	72	♀	25-50	Gravid	
"	72	♂	10-25	Mature	
"	72	♀	25-50	Gravid	
"	72	♀	10-25	Gravid	Eggs did not hatch
"	72	♀	25-50	Gravid	
"	72	♂	25-50	Mature	
"	72	♀	1-10	Gravid	

aquarium partially filled with artificial spring water and fed laboratory-reared meal worm (*Tenebrio molitor*) larvae. Reptilian hosts (Tables 4, 5) were allowed to feed on laboratory-reared meal worms (larvae and adults) until those force-fed experimentally developed metacercariae of *P. minimum*.

Wild birds (Tables 6, 7) were maintained on a variety of crushed grains and water for at least 15 days after capture and before experiments were undertaken. Wild nestlings as well as hatchery-acquired, day-old cockerel chickens were fed metacercariae of *P. minimum* (from naturally infected fish hosts) within 48 hours after hatching. For comparative purposes, adult *P. minimum* were collected from several naturally infected adult piscivorous avian hosts (*Larus delawarensis* and *Sterna forsteri*) from Miller's Bay (Table 8).

Mammalian hosts (Tables 9, 10) were maintained in appropriate cages and held in the laboratory for 30 days where they were examined periodically for the presence of *P. minimum* eggs. All infected hosts were then force-fed metacercariae of *P. minimum*.

EXPERIMENTAL INFECTIONS.— In preliminary experiments, definitive hosts were force-fed sunfish livers naturally infected with over 100 metacercariae of *P. minimum*. Once fed, all hosts were main-

Table 4. Reptilian hosts fed sunfish livers naturally infected with metacercariae of *P. minimum*.

Hosts	Age of infection (hrs)	Host sex	Number of specimens recovered	State of sexual maturity
Order: Chelonia				
Chrysemys picta	84	♂	25-50	Mature
"	96	♀	100+	Mature
Order: Squamata				
Thamnophis radix	48	♀	50-100	Mature
Thamnophis sirtalis	24	♀	100+	Gravid
"	48	♀	100+	Mature

Table 5. Reptilian hosts fed sunfish livers experimentally infected with metacercariae of *P. minimum*.

Hosts	Age of infection (hrs)	Host sex	Numbers of specimens recovered	State of sexual maturity	Laboratory maintenance of life cycle
Order: Chelonia					
Chrysemys picta	48	♀	1-10	Gravid	
"	72	♀	1-10	Mature	
"	72	♂	1-10	Gravid	Egg Cercariae
Order: Squamata					
Thamnophis radix	48	♀	100+	Mature	
"	72	♀	100+	Mature	
"	72	♀	25-50	Mature	
"	72	Immature	1-10	Mature	
"	72	Immature	1-10	Gravid	
Iguana iguana	48	♂	1-10	Mature	
"	72	♂	0	0	

tained in appropriate cages or aquaria and fed only water. After a suitable developmental period of 22-96 hours, these hosts were examined for the presence of *P. minimum* adults, using standard routine laboratory methods.

Three eggs of *Posthodiplostomum minimum*, obtained from a single gravid worm in an experimentally infected chicken 48 hours postexposure, were placed in an embryological watch glass with millipore-filtered lake water. Hatching of the miracidia occurred 20-21 days later.

A single miracidium was exposed to a laboratory-reared *Physa gyrina* and penetration was observed. This snail was isolated in a one-gallon aquarium and maintained as previously stated. Shedding of cercariae took place approximately 48 days postpenetration. Twice daily for 10 days contents of the one-gallon aquarium were poured into an aquarium containing laboratory-maintained sunfish (mentioned above). Sunfish were then maintained at room temperature for 45 days, after which the temperature of the water was gradually reduced to 38 F. These sunfish livers served as the source of metacercariae for subsequent experimental feedings to definitive hosts. Gravid *Posthodiplostomum minimum* from such experimental feedings provided eggs for maintenance of the life cycle in the laboratory.

All definitive hosts which had been exposed to laboratory-developed metacercariae were autopsied 49 to 96 hours postinfection. Adult worms so obtained were washed in the appropriate Ringer's solution and were prepared for light microscopy, scanning, or direct electron microscopy.

Microscopy.— Specimens for whole-mount preparation were doubly washed in the appropriate Ringer's solution and fixed in warmed 10% neutral buffered formalin solution. Specimens were then dehydrated in ethanol, stained in Mayer's paracarmine, counterstained with fast green, cleared in methyl salicylate, and mounted in Permount. In no instances were specimens flattened with coverslip pressure.

All worms were accurately measured with a calibrated ocular micrometer.

Specimens for direct electron microscopy were prepared according to methods published by Lumsden (1970).

TABLE 6. Avian hosts fed sunfish livers naturally infected with metacercariae of *P. minimum*.

Hosts	Age of infection (hrs)	Host sex	Numbers of specimens recovered	State of sexual maturity	
ORDER: GALLIFORMES					
Gallus domesticus	42	♂	25-50	Gravid	
"	24	♂	100	Mature	
"	48	♂	100	Gravid	
"	48	♂	25-50	Gravid	
"	36-42	♂	25-50	Gravid	
"	36-42	♂	50-100	Gravid	
"	36-42	♂	25-50	Gravid	
"	36-42	♂	50-100	Gravid	
"	72	♂	50-100	Gravid	Egg Cercariae
"	24	♂	100	Mature	
"	36-42	♂	50-100	Gravid	
"	72	♂	25-50	Gravid	
"	72	♂	50-100	Gravid	
"	72	♂	1-10	Gravid	
Meleagris gallopavo	36	♂	25-50	Mature	
ORDER: PASSERIFORMES					
Passer domesticus	48	Immature	1-10	Mature	
"	48	Immature	0	Control	
"	26	♂	0	0	
"	48	♀	0	0	
"	28	♂	0	0	
"	48	♂	1-10	0	
"	48	♂	1-10	Immature	
"	48	♂	1-10	Mature	
"	40	?	1-10	Mature	
Parus atricapillus	22	♀	1-10	Mature	
Pheucticus ludovicianus	54	♂	100+	Gravid	
Cyanocitta cristata	24	♂	25-50	Mature	
"	48	♂	25-50	Mature	
"	54	♂	0	0	
Toxostoma rufum	42	♀	1-10	Gravid	
"	28	♂	50-100	Mature	
Troglodytes aedon	38	Immature	10-25	Gravid	
"	38	Immature	0	Control	
"	24	♂	10-25	Mature	
Quiscalus quiscula	72	♂ Immature	0	0	
"	48	♂	0	0	
Turdus migratorius	36	♂	1-10	Mature	
ORDER: PICIFORMES					
Colaptes auratus	24	♂	100+	Mature	
ORDER: COLUMBIFORMES					
Streptopelia risoria	48	♀	1-10	Gravid	
"	48	♀	50-100	Gravid	
"	48	♂	25-50	Gravid	
"	48	♂	0	0	
"	48	♀	0	0	
Streptopelia risoria	48	♀	0	0	
"	48	♂	0	0	
"	48	♂	0	0	
Zenaidura macroura	24	♀	1-10	Mature	
ORDER: ANSERIFORMES					
Anas platyrhynchos	24	♀	1-10	Gravid	
"	48	♀	1-10	Gravid	
"	48	♀	1-10	Gravid	
"	48	♀	50-100	Gravid	
"	48	♂	1-10	Gravid	
"	48	♂	10-25	Gravid	

Table 7. Avian hosts naturally infected with adults of *P. minimum.*

Host	Host sex	Number of specimens recovered	State of sexual maturity
Order: Charadriiformes			
Larus delawarensis	♂	1-10	Gravid
Larus delawarensis	♂	10-25	Gravid
Sterna forsteri	♂	1-10	Mature
Order: Ciconiiformes			
Ardea herodias	?	10-25	Gravid

Table 8. Avian hosts fed sunfish livers experimentally infected with metacercariae or *P. minimum.*

Hosts	Age of infection (hrs)	Host sex	Numbers of specimens recovered	State of sexual maturity	Laboratory maintenance of life cycle
Order: Galliformes					
Gallus domesticus	96	♂	100+	Gravid	Egg Cercariae
"	96	♂	100+	Gravid	Egg Cercariae
"	96	♂	1-10	Gravid	Egg Cercariae
"	96	♂	1-10	Gravid	Egg Cercariae
"	96	♂	1-10	Gravid	Egg Cercariae
"	96	♂	25-50	Gravid	Egg Cercariae
"	96	♂	100+	Gravid	Egg Cercariae
"	96	♂	100+	Gravid	Egg Cercariae
"	96	♂	100+	Gravid	Egg Adult
Meleagris gallopavo	48	♂	25-50	Mature	Cercariae
"	48	♂	25-50	Mature	
Order: Passeriformes					
Quiscalus quiscula	72	♂	0	0	
"	72	♂	0	0	
"	72	♂	0	0	
"	72	♂	0	0	
Richmondena cardinalis	72	♂	0	0	
"	72	♀	0	0	
Order: Charadriiformes					
Larus argentatus	72	♂	100+	Gravid	Egg Egg
Order: Columbiformes					
Streptopelia risoria	48	♂	1-10	Gravid	
"	48	♀	1-10	Mature	
"	48	♀	1-10	Gravid	Egg Adult Egg
Columba livia	48	♂	25-50	Gravid	
"	72	♂	1-10	Gravid	
"	72	♂	1-10	Gravid	
"	72	♂	1-10	Gravid	
"	72	♂	0	0	
Zenaidura macroura	72	♂	1-10	Gravid	

Specimens to be examined by scanning electron microscopy were fixed in a modified Parducz (1967) solution (6.0 ml of 2% O_8O_4 and 1.0 ml of saturated mercuric chloride) for one minute at OC. All specimens were then washed in distilled water three times at 15-minute intervals. Entire specimens were rapidly dehydrated in ethanol using critical-point drying techniques as described by Hearle, Sparrow, and Cross (1972), Cohen and Shaykh (1973), Polliack, Lampen, and de Harven (1973), and Lewis and Nemanic (1973).

Dried specimens were then affixed by electrically conductive aluminum paint to cleaned brass plates and secondarily affixed to brass specimen holders. Specimens were initially coated with carbon and were subsequently given a double coat of gold-palladium. All specimen coating was done with the aid of an Edwards vacuum evaporator. Coated specimens were viewed and photographed on a Jeolco JSM-S1 scanning electron microscope at an accelerating voltage of either 4 or 10 KV. All micrographs were recorded on Kodak Ektapan 4162 negative film and developed in a mixture of six parts Kodak D-76 and one part Kodak

TABLE 9. Mammalian hosts fed sunfish livers naturally infected with metacercariae of *P. minimum*.

Hosts	Age of infection (hrs)	Host sex	Numbers of specimens recovered	State of sexual maturity
ORDER: RODENTIA				
Citellus tridecemlineatus	72	♂	0	0
Peromyscus leucopus	22	♀	10-25	Mature
"	36	♀	0	0
"	28	♂	1-10	Mature
Tamiasciurus hudsonicus	72	♀	0	0
Tamias striatus	42	♂	0	0
"	44	♂	1-10	Mature
"	48	Immature	1-10	Mature
Ondatra zibethicus	48	♀	100+	Gravid
ORDER: LAGOMORPHA				
Oryctolagus cuniculus	48	♂	25-50	Gravid
ORDER: INSECTIVORA				
Blarina brevicauda	57	♂	1-10	Mature
"	22	♂	100+	Mature
ORDER: MARSUPIALIS				
Didelphis marsupialis	36	♀	100+	Gravid
"	48	♀	100+	Gravid
ORDER: CARNIVORA				
Mustela erminea	24	♂	50-100	Mature

TABLE 10. Mammalian hosts fed sunfish livers experimentally infected with metacercariae of *P. minimum*.

Hosts	Age of infection (hrs)	Host sex	Number of specimens recovered	State of sexual maturity	Laboratory maintenance of life cycle
ORDER: RODENTIA					
Mus musculus	72	♂	1-10	Mature	
"	72	♀	0	0	
"	72	♂	0	0	
"	72	♀	0	0	
"	72	♀	0	0	
Meriones unguiculatus	48	♂	1-10	Gravid	
"	72	♀	0	0	
"	72	♂	10-25	Gravid	
"	72	♀	25-50	Gravid	
"	72	♂	25-50	Gravid	
"	72	♂	25-50	Gravid	
Citellus tridecemlineatus	72	♂	0	0	
"	72	♂	0	0	
"	72	♀	0	0	
"	72	♀	0	0	
ORDER: LAGOMORPHA					
Oryctolagus cuniculus	72	♂	1-10	Gravid	
"	72	♂	1-10	Gravid	
"	72	♂	0	0	
ORDER: CARNIVORA					
Felis catus	72	♂	25-50	Gravid	Eggs Miracidium
"	72	♂	25-50	Gravid	
Canis familiaris	72	♂	1-10	Mature	
"	72	♂	1-10	Mature	

D-19 for maximum resolution and negative contrast.

RESULTS

Adult worms recovered during this investigation were derived from two sources, namely: (1) sunfish livers naturally infected with metacercariae of *P. minimum* and (2) feeding sunfish livers experimentally infected with metacercariae of *P. minimum* to experimentally reared definitive hosts. In all experiments intermediate and definitive hosts were in-

fected with life cycle stages derived from one of the above sources of *P. minimum*. In this study the former source will be considered the natural line; the latter, the experimental line.

Natural line of infection.— Beginning June 1971 and continuing to January 1974, detailed experimental feedings as well as examinations of local vertebrate hosts were carried out at Iowa Lakeside Laboratory and Iowa State University. During this period well over 250 possible amphibian, reptilian, avian, and mammalian definitive hosts of the West Lake Okoboji region were examined, and two previously unrecorded species of natural avian hosts for adult *P. minimum* were found (Palmieri 1973). In toto 114 vertebrate hosts were fed naturally infected sunfish livers containing metacercariae of *P. minimum*; 60 proved to be suitable hosts for adult worms (Tables 11, 12). In no cases were adult *P. minimum* recovered from fish hosts, although after 96 hours of exposure, actively moving encysted metacercariae were still found within the intestine of 12 of 32 exposed piscine hosts (Table 13).

Natural snail populations in the Miller's Bay marsh area include two species of snails (*Physa gyrina* and *Lymnaea reflexa*) capable of producing cercariae of *Posthodiplostomum minimum* similar to those reported by Miller (1954).

Several day-old chickens were fed livers infected with neascus-type metacercariae of *P. minimum* (over 2,000 metacercariae per chicken), and fecal samples were checked periodically for the presence of eggs. When *P. minimum* eggs were recovered in the feces, cockerels were dissected and fluke eggs collected from both gut and fecal materials. Several hundred eggs were washed and isolated in small embryological watch glasses and covered with filtered lake water. Eggs were incubated at 21 C and were observed four times daily for the presence of hatched

Table 11. Total number of vertebrate hosts exposed to livers naturally infected with metacercariae of *P. minimum*.

Class	Positive	Negative	No. species	Total no. hosts
Fish	0	32	4	32
Amphibians	3	1	3	4
Reptiles	5	0	3	5
Birds	41	17	15	58
Mammals	11	4	9	15
Total	60	54	34	114

Table 12. Total number of vertebrate hosts exposed to fish livers experimentally infected with metacercariae of *P. minimum*.

Class	Positive	Negative	No. species	Total no. hosts
Amphibians	17	4	4	21
Reptiles	8	2	3	10
Birds	18	9	8	27
Mammals	13	9	6	22
Total	56	24	21	80

Table 13. Fish hosts exposed to sunfish livers naturally infected with metacercariae of *P. minimum*.

Fish host	Common name	Age of infection (hrs)	Number of fish demonstrating: Unexcysted metacercariae (+)	Unexcysted metacercariae (–)	Adults of *P. minimum*
Micropterus dolomieu dolomieu Lacepede	Northern Smallmouth Bass	96	4	4	0
Micropterus salmoides (Lacepede)	Largemouth Bass	96	0	8	0
Lepomis gibbosus (Linnaeus)	Pumpkinseed	96	8	0	0
Perca flavescens (Mitchill)	Yellow Perch	96	0	8	0

miracidia. Hatching took place beginning day 21 and continuing through day 24. During the same period additional eggs of *P. minimum* were placed in two one-gallon aquaria (over 500 eggs per aquarium), one containing 12 laboratory-raised *P. gyrina* and the other a similar number of laboratory-reared *L. reflexa*. Aquaria were checked daily for the presence of cercariae. Emergence of cercariae from *P. gyrina* began on day 47 and on day 58 from *L. reflexa*. Because more cercariae emerged from *P. gyrina* and appeared more active, *P. gyrina* was employed as the experimental first intermediate host.

As previously stated, two lines of *P. minimum* exist, one line found in centrarchid fish and another in cyprinid fish. Examination of sunfish from Miller's Bay indicated that all specimens collected were positive for metacercariae of *P. minimum* (Table 1). Fry usually demonstrate a lighter infection rate (100-500 metacercariae), whereas older fish commonly contain from 500 to 2,000 metacercariae. The intensity of infection in sunfish appears to be due to size of the fish rather than to density of snails shedding cercariae of *P. minimum*. This fact confirms similar earlier reports by Klak (1940), Hoffman (1953, 1958), Colley and Olson (1963), and Avault and Allison (1965). In detailed examinations of the viscera, the liver appears to be the most heavily infected organ, but spleen, heart, kidney, mesentaries, and the surface of major blood vessels also are sites of encystation of *P. minimum* metacercariae. In no other fish examined from Miller's Bay (Table 1) were metacercariae of *P. minimum* ever found. This evidence clearly shows sunfish to be the major source of natural infections of *P. minimum* in piscivorous birds of the Okoboji region. This finding strongly supports statements by Klak (1940), Hunter and Hunter (1940), Ferguson (1943), Hoffman (1960), and Bedinger and Meade (1967) that a distinct centrarchid line of *P. minimum* does indeed exist.

Exposure of livers of sunfish containing naturally infected metacercariae of *P. minimum* to a variety of vertebrate hosts established 21 new experimental host records including 34 individual species representing 15 orders within 4 classes of vertebrates. A complete summary of all feeding experiments can be found in Table 11.

During examination of the vertebrates inhabiting the Miller's Bay area, two gulls (*Larus delawarensis*) and one tern (*Sterna forster*) were found to be naturally infected with mature or gravid adult *Posthodiplostomum minimum* (Table 8). All had been observed feeding on fish in Miller's Bay before collections were made, and both species represent new host records.

Gravid *P. minimum* were recovered from all four classes of vertebrate hosts exposed, although egg size varied greatly among specimens from them. Egg numbers per worm varied from one to five, depending on the experimental host utilized.

Much variation exists in localization of adults as well as in their density in experimental definitive hosts. In poikilothermic groups (amphibians and reptiles) adult *P. minimum* were found principally at the extreme anterior and posterior regions of the intestine. One exception to this was in *Ambystoma tigrinum*, in which adults were distributed throughout the intestine. One female *Rana pipiens* had mature *P. minimum* developing within the stomach 96 hours after infection. In two species of snakes (*Thamnophis radix* and *T. sirtalis*) the most highly developed worms were found in the anterior third of the intestine. This variation in site localization also held true for worms recovered from two specimens of turtles (*Chrysemys picta*). Among homoiothermic hosts examined, site localization of adult *P. minimum* varied greatly from those of poikilothermic hosts. Most *P. minimum* adults recovered from the following avian hosts were recovered from the upper third to upper half of the small intestine: *Gallus domesticus*, *Cyanocitta cristata*, *Toxostoma rufum*, *Colaptes auratus*, *Meleagris galippavo*, and *Anas platyrhynchos*. In six species of avian hosts (*Passer domesticus*, *Larus delawarensis*, *Zenaidura macroura*, *Troglodytes aedon*, *Columba livia*, and *Streptopelia risoria*) adult worms were found only in the midregion of the small intestine. In a few instances exceptions to the above site localizations were noted: *Sterna forsteri* (junction of the small and

large intestine), *Troglodytes aedon* (midsmall intestine, liver and lungs), *Turdus migratorius* (throughout the intestinal tract), and *Pheucticus ludovicianus* (esophagus and throughout the digestive tract). Less variation in site localization was noted in mammalian definitive hosts. Here, localization varied from extreme upper six inches of the small intestine (*Oryctolagus cuniculus*) to the upper third to anterior half (*Didelphis marsupialis*, *Blarina brevicauda*, and *Tamias striatus*). In several hosts (*Mustela erminea*, *Peromyscus leucopus*, and *Ondatra zibethicus*) adult *P. minimum* was limited to the midregion of the small intestine.

Pathology.— Sunfish collected from an area free from *P. minimum* infection were exposed twice daily to over 500 cercariae for a period of 10 days. Very little irritability resulting from cercarial penetration of the sunfish was observed. These results are in agreement with those reported by Klak (1940) and Sillman (1957).

Development of metacercariae was allowed to take place within the fish host for a minimum of 45 days before experimental feedings were begun. Examination of the experimentally infected sunfish indicated that site localizations of metacercariae were similar to those seen in naturally infected sunfish from Miller's Bay, but the density of infection was much reduced. Most fish contained between 75 and 300 metacercariae, with the greatest numbers occurring within the liver.

Very little information exists concerning the pathology of adult *P. minimum* in the definitive host. During this investigation no apparent ill effects were observed due to infections of *P. minimum*. In no case did any poikilotherm show any evidence of pathology due to an infection of this fluke. Some effects, however, were noted for homoiothermic hosts.

Avian hosts infected with large numbers of worms (above 200) showed signs of enteritis and diarrhea. Some destruction of intestinal papillae and blood vessels as well as petechial and catarrhal enteritis occurred. No avian host was ever lost due to infection by *Posthodiplostomum minimum*, even in instances where over 2,000 adult worms were collected from a three-day-old *Gallus domesticus* and adult *Cyanocitta cristata*.

Mammalian hosts showed the greatest range of pathology resulting from infection by *P. minimum*. Effects ranged from no apparent harm to complete destruction of most of the villi of the upper third of the small intestine. In an opposum (*Didelphis marsupialis*) extreme hemorrhagic enteritis was noted within the intestine of a pregnant female.

Viability.— Gravid adults were recovered from all four classes of vertebrate hosts which had been fed laboratory-raised metacercariae of *P. minimum* (Tables 2-10). Several attempts were made to determine the viability of eggs collected from these hosts, and attempts were made to maintain the life cycle in the laboratory.

Eggs from gravid worms which had developed in a single female *Rana pipiens* failed to develop after 40 days incubation. No other attempt was made to show viability in amphibian hosts. Eggs from a turtle host (*Chrysemys picta*) developed, hatched, and miracidia penetrated a single *P. gyrina*. Development proceeded to the point of cercarial emergence, but further attempts to continue the life cycle were not undertaken. Attempts at hatching eggs taken from adult worms raised in a young male cat (*Felis catus*) proved successful up to the free-swimming miracidial stage. Greatest success in maintenance of the life cycle of *P. minimum* in the laboratory, however, was found within the class Aves. Attempts to hatch eggs and to develop free-swimming cercariae from eggs collected from adult *P. minimum* reared in nine-day-old *Gallus domesticus* proved successful. Fully developed infective metacercariae reared in sunfish were fed to day-old cockerel chicks and gravid adult *Posthodiplostomum minimum* were recovered in 36 hours. Eggs were then hatched, miracidia exposed to laboratory-reared snails, and development observed up to the cercarial stage. A similar cycle (egg to egg) was also carried out using a domesticated dove (*Streptopelia risoria*) (Table 7).

Site localization of adult *P. minimum* experimentally developed in vertebrate hosts was found to be similar to that in

hosts which had been fed naturally infected sunfish livers.

Discussion

The strong specificity supposedly demonstrated by strigeoids has been the basis for several extensive taxonomic revisions of this group by Dubois (1944, 1955, 1968, 1970). In recent years, however, several investigators have shown that strigeoid trematodes are not physiologically as host specific as previously suggested. Ulmer (1961) emphasized the need for additional experimental data relative to host specificity, in order to assess the validity of Dubois' use of it as a major criterion for establishing taxonomic relationships.

Berrie (1960) and others have stated that new species of parasites are often described on the basis of a very few specimens recovered from a single host individual. In such circumstances an overemphasis is placed on apparent host specificity. The large number of so-called "species" assigned to a given genus unfortunately results in taxonomic confusion. This is particularly true insofar as the genus *Plagiorchis* is concerned, for more than 90 described species appear in the literature. Angel (1959) called attention to the increasing problems resulting from the burgeoning numbers of species in that genus, and concluded that increasing difficulty would result "unless authors will appreciate the necessity of allowing for some considerable amount of variation of characters within a species . . . and for the possibility that some species may occur in a more or less wide range of hosts."

This investigation clearly demonstrates that *Posthodiplostomum minimum* is able to develop to a gravid state in many host species within all vertebrate classes except fishes. It is doubtful, however, that host specificity in a strict sense is of value in differentiating species of *Posthodiplostomum*. Most definitive hosts utilized during this investigation probably would not be found naturally infected with *P. minimum*. Ecological isolation and other factors prevent many hosts from actively feeding upon infective metacercariae within the fish intermediate host. Nonetheless, accidental infections could occur and clearly indicate that host specificity of strigeoids as a major taxonomic criterion is apt to be unreliable.

Because the adult stage of *P. minimum* is capable of developing in a variety of vertebrate hosts and the larval stages employ numerous intermediate hosts, little value can be placed upon host specificity as a major taxonomic tool. Because of specificity and the striking plasticity of body shape and size and organ shape, size, and position, it is indeed probable that many of the reported species of *Posthodiplostomum* are one and the same and should be placed in synonomy with one another. Experimental data of the type analyzed during this investigation emphasize the need for an extensive and complete revision of the genus *Posthodiplostomum* as well as the necessity for experimental determination of relationships between species of *Posthodiplostomum* and their reported definitive hosts. Work in this area will require a more flexible interpretation of the species taxon and should provide us with a more meaningful relationship between *Posthodiplostomum* and its hosts.

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